

We Claim:

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1. A method for the production of chymosin in a plant seed comprising:
- 5 sequence molecule comprising in the 5' to 3' direction of transcription:
- 1) a first nucleic acid sequence capable of regulating transcription in said plant cell operatively linked to;
 - 2) a second nucleic acid sequence encoding a chymosin polypeptide operatively linked to;
 - 10 3) a third nucleic acid sequence capable of terminating transcription in said plant cell;
- b) growing said plant cell into a mature plant capable of setting seed; and
- c) obtaining seed from the mature plant wherein said seed
- 15 contains chymosin.
2. The method according to claim 1 wherein said first nucleic sequence capable of regulating transcription in said plant cell is a seed-specific promoter.
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3. The method according to claim 3 wherein said seed-specific
- 20 promoter is a phaseolin promoter.
4. A method according to claim 1 wherein at least 0.5% (w/w) of the total seed protein is chymosin.
5. The method according to claim 1 wherein the second nucleic acid sequence encoding a chymosin polypeptide comprises a nucleic acid
- 25 sequence encoding a chymosin pro-peptide, a nucleic acid sequence encoding a chymosin pre-peptide or a nucleic acid sequence encoding chymosin pre-pro-peptide.

6. The method according to claim 5 wherein the second nucleic acid sequence encoding a chymosin polypeptide further comprises a nucleic acid sequence encoding a plant signal sequence.

7. The method according to claim 1 wherein the second nucleic acid sequence encoding a chymosin polypeptide further comprises a nucleic acid sequence encoding a plant signal sequence.

8. ~~The method according to claim 7 wherein the plant signal sequence is a tobacco PR-S sequence.~~

9. The method according to claim 8 wherein the nucleic acid sequence encoding chymosin linked to a PR-S signal sequence comprises a nucleic acid sequence as in SEQ.ID.NO.:1.

10. The method according to claim 1 wherein said third nucleic acid sequence is a phaseolin terminator.

11. ~~The method according to claim 1 wherein the chymosin is a mammalian chymosin obtainable from a bovine, sheep or goat source.~~

12. The method according to claim 6 wherein codon usage for said nucleic acid sequence encoding chymosin, chymosin pro-peptide, chymosin pre-peptide and chymosin pre-pro-peptide has been optimized for use in plants.

13. The method according to claim 1 wherein said plant is selected from the group of plants consisting of soybean (*Glycine max*), rapeseed (*Brassica napus*, *Brassica campestris*), sunflower (*Helianthus annuus*), cotton (*Gossypium hirsutum*), corn (*Zea mays*), tobacco (*Nicotiana tabacum*), alfalfa (*Medicago sativa*), wheat (*Triticum sp.*), barley

(*Hordeum vulgare*), oats (*Avena sativa* L.), sorghum (*Sorghum bicolor*),
Arabidopsis thaliana, potato (*Solanum sp.*), flax/linseed (*Linum
usitatissimum*), safflower (*Carthamus tinctorius*), oil palm (*Eleais
guineensis*), groundnut (*Arachis hypogaea*), Brazil nut (*Bertholletia excelsa*)
5 coconut (*Cocos nucifera*), castor (*Ricinus communis*), coriander
(*Coriandrum sativum*), squash (*Cucurbita maxima*), jojoba (*Simmondsia
chinensis*) and rice (*Oryza sativa*).

14. The method according to claim 1 wherein at least 1% (w/w) of
said total seed protein is chymosin.

10 15. The method according to claim 1 wherein at least 2% (w/w) of
said total seed protein is chymosin.

16. The method according to claim 1 wherein at least 4% (w/w) of
said total seed protein is chymosin.

34 17. A method for the production of plant seeds containing at least
15 0.5% (w/w) chymosin in the total seed protein comprising:

(a) introducing into each of at least two plant cells a chimeric
nucleic acid sequence molecule comprising in the 5' to 3' direction of
transcription:

- 20 1) a first nucleic acid sequence capable of regulating
transcription in said plant cell operatively linked to;
2) a second nucleic acid sequence encoding a chymosin
polypeptide operatively linked to;
3) a third nucleic acid sequence capable of terminating
transcription in said plant cell;
- 25 (b) growing each plant cell into a mature plant capable of
setting seed;
- (c) obtaining seed from each mature plant;

(d) detecting the levels of chymosin in the seed of each plant obtained in step (c) or in the seed of a plant generated from the seed of a plant obtained in step (c); and

5 (e) selecting plants that contain at least 0.5% (w/w) chymosin in the total seed protein.

18. A method according to claim 1 further comprising (d) isolating said chymosin from said seed.

19. A method according to claim 18 wherein (d) isolating said chymosin from said seed comprises:

- 10 (i) crushing the plant seed to obtain crushed plant seed;
(ii) contacting the crushed plant seed or a fraction thereof with a protein binding resin; and
(iii) recovering chymosin from the protein binding resin.

20. A method according to claim 18 wherein (d) isolating said
15 chymosin from said seed comprises:

- (i) crushing of the plant seed to obtain crushed plant seed;
(ii) fractionating the crushed plant seed into an oil fraction, aqueous fraction and a fraction comprising insoluble material;
20 (iii) contacting the aqueous fraction with a protein binding resin; and
(iv) recovering the chymosin from the protein binding resin.

21. A method according to claim 19 wherein said protein binding resin is a hydrophobic interaction resin.

25 22. A method according to claim 20 wherein said protein binding resin is a hydrophobic interaction resin.

23. A method according to claim 22 further comprising using an ion exchange resin to further purify the chymosin.

24. Plant seed comprising at least 0.5% (w/w) heterologously expressed chymosin.

5 25. Plant seed prepared according to the method of claim 1.

26. Plant seed prepared according to the method of claim 17.

27. A plant capable of setting seed comprising at least 0.5% (w/w) of heterologously expressed chymosin.

10 28. A plant capable of setting seed prepared according to the method of claim 1.